

Protective Immunity against Respiratory Syncytial Virus in Early Life after Murine Maternal or Neonatal Vaccination with the Recombinant G Fusion Protein BBG2Na

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Maternal and neonatal immunization were evaluated for their capacity to induce protective immunity against respiratory syncytial virus (RSV) lower respiratory tract infections in early life. Murine models were studied by use of a novel recombinant vaccine candidate, designated BBG2Na, which was derived in part from the RSV (Long) G protein. Maternal immunization resulted in the passive transfer of high levels of RSV-A antibodies to the offspring, which protected them from RSV challenge for up to 14 weeks. Indeed, protection correlated with the detection of RSV antibodies in the serum. Neonatal immunization with BBG2Na induced significant antibody responses even in the first week of life. Most importantly, these neonatal responses were not inhibited by the presence of RSV maternal antibodies. Consequently, the combination of maternal and neonatal immunization with BBG2Na resulted in the continual presence of protective levels of antibodies in the offspring.

Respiratory syncytial virus (RSV) represents the most common cause of lower respiratory tract infection in young children, most of whom are affected during their first winter season. RSV disease represents a significant cause of infant morbidity, since 0.5%–2% of infected infants require hospitalization at a median age of ≤ 3 months [1–5]. Infant protection from RSV thus represents a major public health issue and a challenge that has not yet been met. An ideal vaccine should induce protective immune responses to most if not all circulating strains of both A and B subgroups of RSV, in spite of the significant heterogeneity of the G glycoproteins [6–8]. In view of peak RSV-induced hospitalization occurring at a median age of 3 months, however, protective immunity needs to be already present in the first weeks and months of life. This period is characterized by immune immaturity and persistence of maternal antibodies able to interfere with infant vaccine responses. Disease enhancement at the time of RSV exposure has also been observed after immunization of seronegative infants with a formaldehyde-inactivated viral vaccine [9, 10]. These obstacles have each interfered with the development of a safe and effective RSV vaccine.

Among the vaccine strategies currently being explored, the recombinant fusion protein BBG2Na has interesting properties. First, it contains an RSV (Long) G protein fragment (G2Na, aa 130–230), including a conserved subgroup A-specific protective epitope [11, 12], and a stretch of amino acid residues (aa 164–176) that are completely conserved in all known human A and B RSV isolates [7, 8]. Second, although not directly demonstrated for G2Na, the albumin-binding region (BB) of streptococcal protein G is capable of significantly enhancing the in vivo half-life of fusion partners [13, 14] and thus their exposure to the immune system; BB also enhances antibody responses to fused peptides [15]. Third, it is produced by prokaryotic expression in *Escherichia coli* as a nonglycosylated protein, thereby circumventing the limitations of the native G protein immunogenicity because of its heavy glycosylation pattern [16]. Last, the strong immunogenicity and protective efficacy of BBG2Na were recently demonstrated against intranasal RSV challenge in both adult BALB/c mice and cotton rats [17]. A potent protective efficacy was demonstrated in mice against both upper and lower respiratory tract RSV subgroup A (RSV-A) infection and was maintained for at least 48 weeks. Importantly, BBG2Na immunization also induced protection against both homologous and heterologous RSV challenge in both adult mice and cotton rats [17].

In this study, we developed a neonatal murine model to assess the capacity of two potential vaccine strategies using BBG2Na to confer early protection against RSV. In a first set of experiments, we asked whether maternal immunization and natural transfer of BBG2Na-induced maternal antibodies was capable of conferring passive protection to the offspring. In a second part of these studies, BBG2Na immunization was performed early in the neonatal period to assess its capacity to induce anti-vaccine or anti-RSV responses (or both) in spite

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Animals were maintained in an accredited facility and experiments were performed in accordance with the guidelines of the local authorities.

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of neonatal immune immaturity and the presence of high levels of either postinfection or vaccine-induced RSV-A maternal antibodies.

Materials and Methods

Mice. Specific pathogen-free adult BALB/c inbred mice were purchased from IFFA-CREDO (L'Arbresle, France) and kept under specific pathogen-free conditions. Breeding cages were checked daily for new births, and the day of birth was recorded as the day the litter was found. Pups were kept with mothers until weaning at the age of 4 weeks.

Vaccine antigen, viruses, and cells. The recombinant fusion protein BBG2Na was expressed in *E. coli* and purified as described [17, 18]. Twenty-microgram doses were used per immunization after resuspension in PBS containing 20% Al(OH)₃ (Alhydrogel; vol/vol; Superfos BioSector, Vedbaek, Denmark) immediately before immunization. RSV-A (Long strain, ATCC VR-26; American Type Culture Collection, Rockville, MD) propagation in HEP-2 cells (ECACC 86030501; European Collection of Animal Cell Cultures, Porton Down, UK), along with the production of viral and control cell ELISA antigens, were as described [18].

Immunization and challenge procedures. Groups of 5–8 mice were immunized intraperitoneally at the ages indicated in the figure legends. They were bled at regular intervals to determine vaccine- and RSV-specific serum antibody titers. When indicated, a booster immunization was given after an interval of 3 weeks. For maternal immunization, two vaccine doses were given at a 3-week interval before mating. Offspring were challenged with 10⁵ TCID₅₀ of RSV-A by intranasal instillation after anaesthetizing with 2.5 mL/kg of a 4/1 mixture (vol/vol) of ketamine (Imalgene 500; Rhône Mérieux, Lyon, France) and xylazine (Rompun 2%; Bayer, Puteaux, France). Challenged mice were sacrificed 5 days after challenge, coincident with previously characterized peak RSV lung titers [19].

Quantification of vaccine- and RSV-specific antibodies. BBG2Na- and RSV-A-specific IgG and subclass antibodies were determined by ELISA. Briefly, flat-bottomed wells of microtiter plates (MaxiSorp; Nunc, Roskilde, Denmark) were coated overnight at 4°C with 50 µL of a 4 µg/mL solution of BBG2Na in PBS or at 37°C and low humidity with 50 µL of RSV-A or HEP-2 cell protein preparations previously calibrated according to their reactivity with relevant reference sera. After washing with PBS–0.05% Tween 20 and blocking with 4% milk solids, serial dilutions of sera were added and incubated for 2 h at room temperature. The relevant isotype-specific peroxidase-conjugated goat or rabbit anti-mouse (Zymed Laboratories, San Francisco) was added for 2 h at 37°C before incubation with substrate. Optical density results were expressed as antibody titers by reference to a titrated reference serum representing a pool of sera from immunized adult mice. As the virus stocks contained cellular antigen, anti-RSV-A-specific antibody titers of mice infected with RSV-A were calculated by subtracting anti-HEP-2 optical density values from those of anti-RSV-A.

Animal sample preparation and virus titration. Animals were anesthetized as described above and exsanguinated by cardiac puncture. Lung removal, lung homogenate preparation, and virus titration in the homogenates were done as previously described

[17]. The limit of detection for lung tissues was $\leq 1.45 \log_{10}$ TCID₅₀/g of lung, except where insufficient lung homogenate was available. When no virus was detected, actual detection limits were used for statistical analyses. Thus, standard deviations >0 were occasionally recorded for lung titers of some virus-free animal groups. Lungs were considered protected when virus titers were reduced by at least 2 log₁₀ relative to control mice.

Statistical analysis. Unless otherwise indicated, significance analyses between results obtained from various groups of mice were done (Stat Graphic; Manujistics, Rockville, MD) by use of the Wilcoxon rank test. $P > .05$ was considered insignificant.

Results

Efficient transfer of BBG2Na-induced antibodies from mothers to offspring. Adult female BALB/c mice were immunized twice with 20 µg of BBG2Na prior to mating. As expected, strong antibody responses to both BBG2Na and RSV-A were observed, often reaching antibody titers $>5.5 \log_{10}$ (figure 1A). When antibody determination was initially done in 3-week-old pups of such BBG2Na-immunized mothers, BBG2Na and RSV-A antibody titers were always as high as the titers detected in their respective mothers (figure 1A). In contrast, no specific antibodies were detected in pups of nonimmunized or PBS-immunized control mothers (data not shown). Serial bleeding of the offspring indicated the prolonged persistence of antibodies of maternal origin, which progressively declined following a decay slope compatible with a half-life of ~ 2 weeks. RSV-A antibodies could thus be detected for periods of between 12 and 20 weeks, depending on the respective maternal antibody titers (data not shown).

Thus, the strong immunogenicity of BBG2Na allowed the induction of high titers of RSV-A antibodies in adult female BALB/c mice, and the natural transfer of BBG2Na-induced antibodies from mothers to pups resulted in the development of adult-equivalent antibody titers and a prolonged persistence of these maternal antibodies in the offspring.

Passive transfer of BBG2Na antibodies from mother to pup was further characterized by the comparative analysis of the subclass distribution of vaccine-specific antibodies in both immune mothers and their 3-week-old pups (figure 1B). IgM and IgA antibodies were below detection levels in both mothers and offspring (data not shown). In contrast, similar high titers of all BBG2Na antibody IgG subclasses were found in both mothers and pups, indicating an efficient transfer of BBG2Na antibodies of all isotypes.

Protective efficacy of BBG2Na-induced maternal antibodies. To assess whether natural transfer of maternal antibodies was sufficient to confer protection to the offspring of immune mothers, litters from BBG2Na-immune or control mothers were challenged with live RSV-A at various ages between the neonatal period (2 weeks) and adulthood (up to 14 weeks). Age-matched immune and control litters were always challenged simultaneously. RSV-A lung titers measured at the peak of

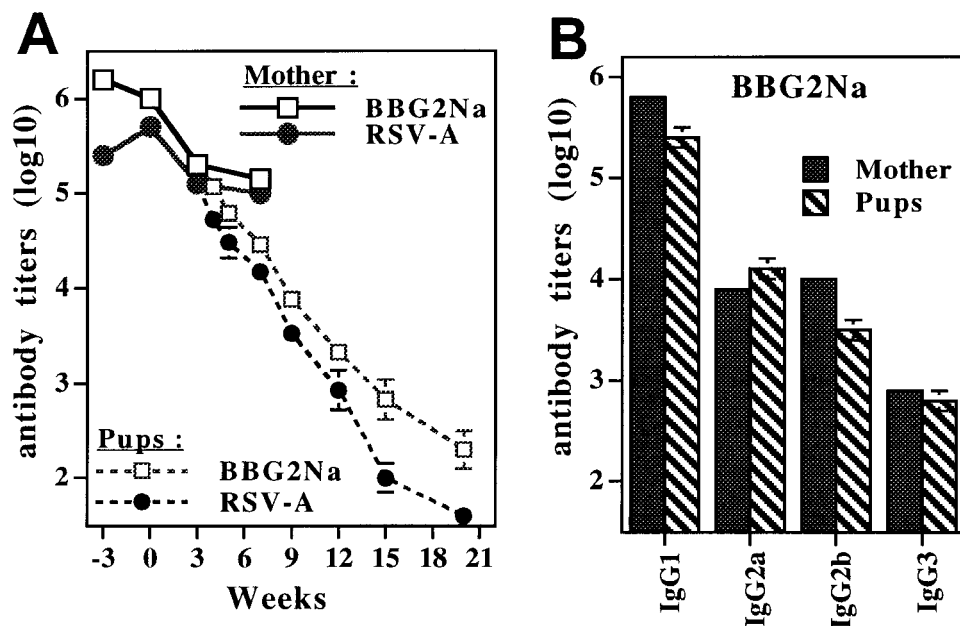


Figure 1. Efficient transfer of BBG2Na-induced maternal antibodies from immune mothers to offspring. Female adult mice were immunized twice at 3-week interval with BBG2Na (20 μ g) before mating. Offspring were kept with mothers up to weaning at 4 weeks of age. Results from representative litter are shown. **A**, BBG2Na- and RSV-A-specific serum antibodies were measured by ELISA at various times (0 = day of birth) in both mother and pups and expressed as mean antigen-specific antibody titers; **B**, IgG isotypes of BBG2Na-specific antibodies were measured by ELISA at time of delivery in mothers and at 3 weeks of age in offspring and expressed as mean vaccine-specific antibody titers.

infection were inversely correlated with the presence of detectable RSV-A maternal antibodies (table 1). High lung virus titers ($>4 \log_{10}$ TCID₅₀/g) were observed in all control mice born to nonimmune mothers, irrespective of age at time of challenge. In contrast, lung protection without detectable virus was observed for all mice in which maternal antibody titers exceeded $4 \log_{10}$. Protection was also observed for all pups of immune mothers challenged at up to 9 weeks of age, although virus was detectable at, or just above, the limit of the assay in an increasing number of mice as their ages progressed and their maternal antibody titers diminished. Surprisingly, significant protection was still observed at 14 weeks of age in 4 of 7 pups whose residual levels of maternal antibodies ($2.0 \log_{10}$) were barely above the cutoff of the ELISA. In contrast, all 5 pups at 14 weeks possessing no detectable residual maternal antibodies were not protected from RSV challenge.

Thus, lung protection from live RSV-A challenge could be achieved early in the neonatal period (2 weeks of age) by BBG2Na maternal immunization and natural transfer of antibodies of maternal origin. Maternal antibody-mediated protection persisted as long as maternal antibodies could be detected in the offspring of immune mothers: for up to 3 months.

Neonatal immunogenicity of BBG2Na. We next wondered whether the strong immunogenicity of BBG2Na would be sufficient to raise significant immune responses in the neonatal period. Immunization was thus initiated in 1- and 2-week-old pups of nonimmune mothers. Interestingly, significant vaccine responses were observed after a single immunization, even in mice that were only 1 week old at time of priming, and reached high antibody levels after a repeat immunization given 3 weeks later (figure 2A).

Comparative analysis of the IgG1 and IgG2a subclass distribution of vaccine-specific antibodies (selected as indirect mark-

Table 1. Protective efficacy of BBG2Na-induced maternal antibodies.

Age (weeks), immunogen to mothers	RSV-A titer (mean \pm SD)		No. virus-free/ no. challenged
	Antibody*	Lung†	
2			
BBG2Na	4.25 \pm 0.1	1.74 \pm 0.38‡	8/8
PBS	<1.4 \pm 0.0	4.00 \pm 0.48	0/5
4			
BBG2Na	5.2 \pm 0.1	<1.46 \pm 0.03§	7/7
BBG2Na	4.8 \pm 0.1	<1.51 \pm 0.13§	4/4
BBG2Na	3.9 \pm 0.1	\leq 1.53 \pm 0.12§	5/7
PBS	<1.4	4.37 \pm 0.38	0/4
7			
BBG2Na	3.8 \pm 0.2	\leq 1.51 \pm 0.13§	1/4
BBG2Na	3.7 \pm 0.2	\leq 1.53 \pm 0.10‡	3/5
PBS	<1.4	4.00 \pm 0.21	0/5
9			
BBG2Na	3.2 \pm 0.1	\leq 1.87 \pm 0.29§	1/3
BBG2Na	3.2 \pm 0.1	\leq 1.53 \pm 0.14‡	4/7
PBS	<1.4	4.30 \pm 0.29	0/5
14			
BBG2Na	2.0 \pm 0.2	\leq 2.20 \pm 0.71§	1/7
BBG2Na	<1.4	3.85 \pm 0.45	0/5
PBS	<1.4	4.20 \pm 0.18	0/5

NOTE. Blood samples were taken from offspring of BBG2Na-immune and control mothers of given age for determination of maternal antibody titers. Pups were challenged through 3 independent experiments assessing simultaneously 2- and 4-, 7- and 9-, and 14-week-old pups. Significance was determined for each group relative to age-matched naive controls.

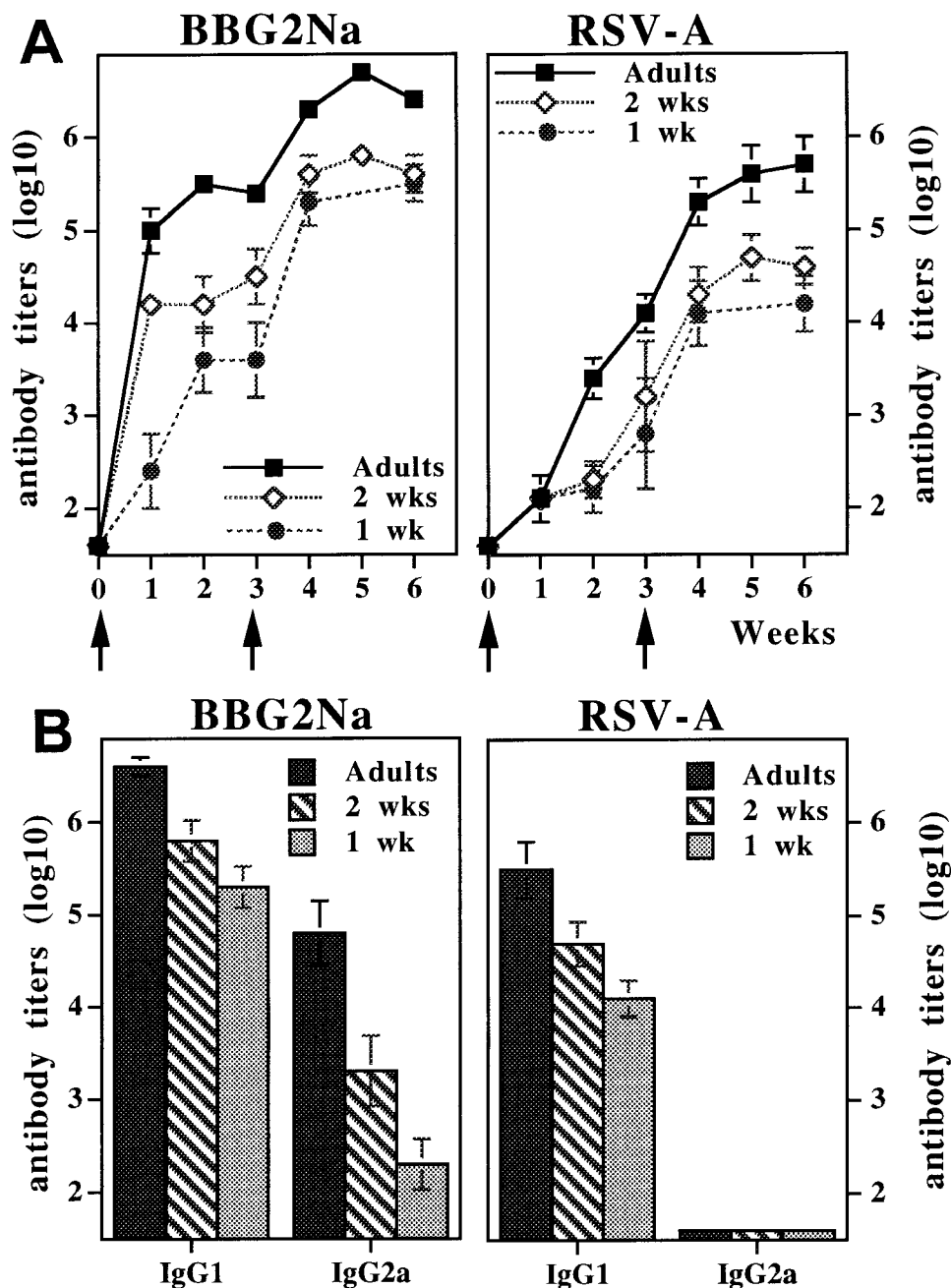
* ELISA titer, \log_{10} .

† RSV-A titer, \log_{10} TCID₅₀/g of lung.

‡ $P < .001$ (t test using null hypothesis).

§ $P < .05$ (nonparametric test; Kolmogorov-Smirnov test because of low sample numbers).

Figure 2. Antibody responses induced by neonatal immunization with BBG2Na. Mice were primed in neonatal period or as adults and boosted 3 weeks later with BBG2Na (20 μ g). **A**, BBG2Na- (left) and RSV-A-specific (right) serum antibodies were measured by ELISA at various times and expressed as mean antigen-specific antibody titers; **B**, IgG1 and IgG2a isotypes of BBG2Na- (left) and RSV-A-specific (right) antibodies were measured by ELISA 1–2 weeks after booster and expressed as mean antigen-specific antibody titers.



ers of Th1/Th2 type of responses) induced by BBG2Na immunization in adult or neonatal mice is reported in figure 2B. As expected in view of our previous observations [20], the IgG2a/IgG1 ratio of BBG2Na-specific antibodies (left) was significantly lower in neonatally compared with adult-primed mice. Thus, the isotype pattern of alum-adsorbed BBG2Na-induced vaccine antibodies is suggestive of a mixed Th1 and Th2 pattern in adult mice and of a Th2 bias of vaccine responses on immunization in early life. However, BBG2Na-induced RSV-A antibodies were found exclusively among the IgG1 and not among the IgG2a isotypes, in both adult- and neonatally primed

mice (figure 2B, right). Thus, IgG2a antibodies are unlikely to play a significant role in the observed protection. Because young mice with passively transferred IgG1 maternal antibodies whose titers were significantly lower ($2.0 \log_{10}$) than those induced by neonatal immunization, even in 1-week-old mice ($>3 \log_{10}$), were protected from RSV challenge (table 1), protection studies were not repeated.

Protective levels of maternal antibodies do not inhibit neonatal vaccine responses to BBG2Na. Since the presence of maternal antibodies is known to exert an inhibitory influence on the induction of vaccine responses in infants, the neonatal im-

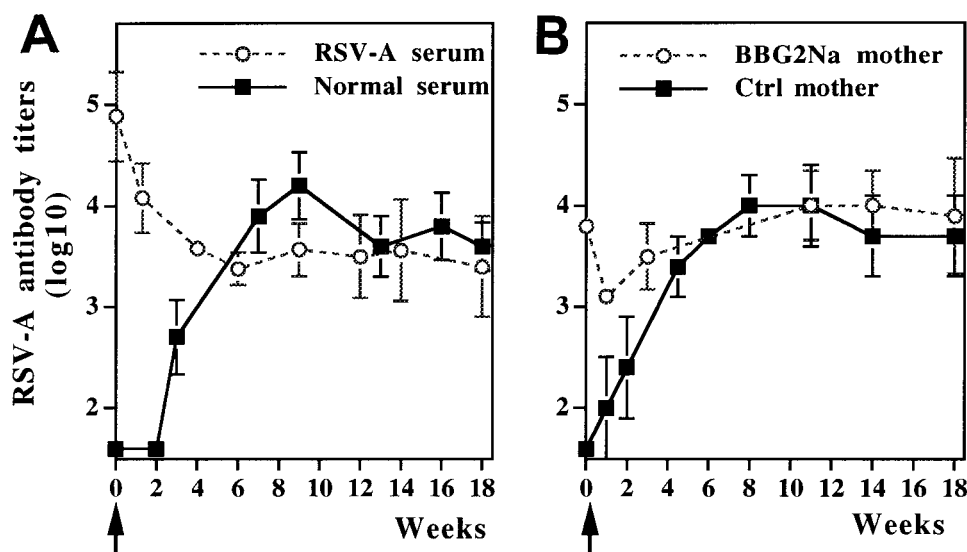


Figure 3. Induction of neonatal responses to BBG2Na despite presence of high titers of maternal antibodies. Mice were immunized at 2 weeks of age with BBG2Na (20 μ g). RSV-A-specific serum IgG antibodies were measured by ELISA at regular intervals and expressed as mean antigen-specific antibody titers. **A**, Immunization was done 48 h after intraperitoneal transfer of postinfection RSV-A-hyperimmune serum (ELISA titer, 5.2 log₁₀) or normal mouse serum; **B**, immunization was performed in pups of either BBG2Na-immune or nonimmune mothers as controls.

munogenicity of BBG2Na was evaluated in both the presence and absence of passively transferred antibodies. In a first set of experiments, we assessed whether passive transfer of high titers of RSV-A antibodies induced by repeated RSV infections would affect the neonatal immunogenicity of BBG2Na. A lot of hyperimmune (5.2 log₁₀) anti-RSV-A polyclonal serum was produced in adult mice by repeat (five times) intranasal instillation of live RSV-A. An aliquot (200 μ L) of this hyperimmune serum was experimentally defined as resulting in approximately mother-like antibody titers after intraperitoneal transfer to 2-week-old mice. Forty-eight hours later, blood samples were taken from these young mice for antibody determination before immunization with a single dose of BBG2Na. In contrast to the natural decay slope of maternal antibodies observed when neonatal immunization was not performed (figure 1A), RSV-A antibodies reached and remained at the same plateau (>3.5 log₁₀) as seen in control mice at all times during the >14-week follow-up period (figure 3A). This observation indicates active production of antibodies by the BBG2Na-immunized pups in spite of the presence of very high titers of passively transferred maternal RSV-A antibodies at time of immunization, and even without administration of a second vaccine dose at a later time point.

In a second set of experiments, we investigated whether BBG2Na would retain its neonatal immunogenicity even in the presence of BBG2Na-induced antibodies resulting from maternal immunization. Offspring from either BBG2Na-immune or control mothers were immunized with a single dose of BBG2Na and followed at regular intervals for antibody determination. Again, when immunization was performed in the presence of a BBG2Na-induced RSV-A titer of maternal antibodies previously demonstrated as fully protective (4 log₁₀, table 1), a single dose of BBG2Na induced the same titer of RSV-A antibodies in pups of either immune or control mothers (figure 3B). Thus, under these experimental conditions, neona-

tal immunization with BBG2Na is not inhibited by the presence of protective titers of vaccine-induced RSV maternal antibodies.

Discussion

This report provides evidence that immunization with BBG2Na before pregnancy results in the passive transfer of high levels of vaccine antibodies to the offspring of immune mothers, such maternal antibodies protect the lungs of the pups against an RSV challenge performed during the neonatal period and up to 14 weeks later, high levels of vaccine-specific antibodies can be induced by BBG2Na even in the first week of life, and such vaccine responses can be induced in the neonatal period in spite of the presence of high levels of RSV maternal antibodies.

A significant proportion of RSV lower respiratory tract infections, including the most severe, occurs in the first 3 months of life. This early occurrence of disease and the observation that RSV antibodies alone can reduce the severity of lung disease (reviewed in [21]) have set the basis for the development of novel vaccine strategies targeting women of childbearing age rather than young infants. The success of such approaches is expected to depend on the capacity of vaccine candidates to induce high and persistent titers of protective RSV antibodies in young women and on the efficacy of placental transfer of maternal vaccine-specific antibodies to infants. These two parameters define the time period during which progressively declining maternal antibodies can be expected to remain above protective levels in infants. Although these properties can be definitively evaluated only by clinical testing in humans, we report here in mice the promising preclinical evaluation of BBG2Na as a candidate for RSV maternal immunization.

The immunogenicity of BBG2Na in adult mice is sufficient to rapidly induce high and persistent levels of RSV-specific antibodies capable of conferring protection against homologous and heterologous RSV challenge in the lower respiratory tract [17]. We show here that the transplacental transfer of BBG2Na-induced antibodies from immune mothers to their offspring is so efficient as to result in the achievement of similar RSV-A-specific antibody levels of all isotypes in mothers and pups. This immunoglobulin transfer through the Fc γ Rn [22, 23] is an active and saturable process that is influenced by several factors in both mice and humans. The first factor is directly related to the level of seric vaccine antibody that can be raised in adults, which favors the use of adjuvanted recombinant RSV proteins or fragments compared with the more local action of intranasally applied live attenuated or mutant viruses. The use of non-live vaccines also offers obvious safety advantages for immunization of women of childbearing age.

The second factor affecting transfer of maternal antibodies is the availability of sufficient time between induction of vaccine antibodies in mothers and delivery. If the prolonged persistence of BBG2Na antibodies (possibly related to the prolonged exposure of the immunogen due to its albumin-binding property) also occurs in humans, it will allow us to consider the immunization of women of childbearing age rather than the more complex approach of vaccination during pregnancy. The third factor is the nature of the vaccine-specific antibodies themselves, defined by both antibody isotypes and other as-yet poorly understood parameters. Here, BBG2Na antibodies of all IgG subclasses were efficiently transferred from mothers to pups. This is in striking contrast to the reduction in maternofetal IgG2b transfer observed in other models of murine maternal immunization analyzed thus far by us (unpublished data) and others [24, 25], including for RSV antibodies [25]. Whether this efficient transfer of all BBG2Na isotypes is linked to their recognition of BB, the albumin-binding component of the immunogen, or of the RSV G2Na fragment will be addressed through maternal immunization studies involving other recombinant fusion proteins.

The protective efficacy in neonates of passively transferred maternal BBG2Na-induced antibodies was similar to that observed after active immunization of adult mice with BBG2Na or RSV-A [17]. It also compared favorably with the protective efficacy of virion-purified [26–29] or recombinant vaccinia virus-expressed RSV G or F proteins or fragments [30–34]. Comparisons between residual maternal antibody titers at various ages and concomitant RSV-A lung virus titers at the time of challenge allowed us to define the antibody levels associated with protection. Complete protection (i.e., no detectable virus 5 days after challenge) was conferred by maternal antibody levels $>4 \log_{10}$. More important, even barely detectable maternal antibody titers ($2.0 \log_{10}$) were sufficient to confer lung protection. These observations demonstrate maternal antibody-mediated protection. They confirm and extend the demonstration of protection following intranasal or intraperitoneal instil-

lation of BBG2Na antibodies in adult mice [17]. The results also underline the strong protective capacity of BBG2Na-induced antibodies, which proved capable of extensive viral clearance at antibody titers considerably below those associated with *in vitro* neutralization activity [17], through yet-undefined molecular mechanisms.

Given the transient nature of protection conferred by maternal immunization, protection from RSV disease in early life is likely to require a sequential approach in which maternal immunization would be followed by the induction of protective immune responses through neonatal immunization. Although this could be achieved through the use of different vaccines, we evaluated the neonatal immunogenicity of BBG2Na. It was found to induce strong vaccine-specific immune responses even in the first week of life, with a 3- to 4-week delay required to reach an antibody titer $>3 \log_{10}$. The previously reported Th2 bias of neonatal vaccine responses [20, 35–38] is also suggested here by the reduced IgG2a/IgG1 ratio of BBG2Na antibodies observed in neonatally primed mice. In view of the concerns related to the induction of Th2-mediated RSV responses in infants, which were linked to disease enhancement at the time of virus exposure [9, 10], we performed a different set of experiments showing that the Th1/Th2 pattern of neonatal vaccine responses to protein antigens depended essentially on the choice of the adjuvant formulation: Selection of a Th1-driving adjuvant could circumvent the preferential Th2 polarization of neonatal responses and induce Th1 responses even in newborn mice if used at time of priming [39]. Similar experiments are pursued with BBG2Na. Given the importance of these concerns, the role of cell-mediated immunity to RSV after BBG2Na immunization in the presence of alum is now being studied in terms of both protection and potential pathologic enhancement.

Passively transferred maternal antibodies have been shown to interfere with the infant capacity to respond to antibody-specific immunogens. Given the ubiquitous nature of RSV and the frequency of reinfections in adults, RSV antibodies of maternal origin are present at low to moderate titers in most infants. Indeed, the reduced antibody response to F and G RSV proteins in infants <9 months of age was shown to result from both immunologic immaturity and maternal antibody-mediated inhibition of vaccine responses [40, 41]. In rodent experimental models, passively transferred RSV antibodies have been reported to interfere with immune responses to intraperitoneal infection with live RSV-A [25, 42], to F- and G-expressing recombinant vaccinia viruses [43], and to F and G glycoproteins [44]. In contrast, we showed here that even high titers ($5 \log_{10}$) of postinfection RSV-A maternal antibodies did not interfere with the induction of antibody responses to a single dose of BBG2Na given at 2 weeks of age. An attractive hypothesis is that the immunogenicity of this nonglycosylated G protein fragment is sufficiently different from the immunogenicity of the native RSV protein equivalent to escape from RSV-A-specific antibody-mediated inhibition. This could be in contrast

to the more closely conserved conformation of the glycosylated G protein either purified from virion [44] or expressed from recombinant vaccinia viruses [43] and reported as susceptible to passive antibody inhibition. Alternatively, the RSV-A fragment of BBG2Na may constitute a relatively poor immunogen in the context of either the whole virion or the native G glycoprotein, such that relatively few G2Na-specific antibodies are induced by the latter immunogens. Indeed, such a hypothesis is consistent with previous reports that showed that RSV-induced BBG2Na-specific antibody titers were in the order of $3 \log_{10}$ [18], while RSV-induced RSV-specific antibody titers were $>5 \log_{10}$ [17] (present study).

However, we also demonstrate in this report that BBG2Na retained its neonatal immunogenicity even when administered to pups possessing levels of maternal antibodies ($4 \log_{10}$) still associated with complete protection (table 1). Thus, a change in conformation of the immunogen or reduced immunogenicity relative to the native protein could not be the sole explanation of the capacity of BBG2Na to escape from maternal antibody-mediated inhibition. Irrespective of the molecular mechanisms involved, however, it is clear that neonatal immunization with BBG2Na resulted in the continual presence of protective levels of antibodies in the offspring of BBG2Na-immune mothers.

In conclusion, this report demonstrated that the combination of murine BBG2Na maternal and neonatal immunization was successful in avoiding the "window of susceptibility" to RSV infection usually present between the transient protection conferred by maternal antibodies and the consequential delayed induction of protective responses in the neonate. For BBG2Na to protect human infants from early RSV disease, it will need to be capable of inducing in adults high and persistent levels of RSV antibodies that are easily transferred from immunized mothers to their infants and remain protective for a few months after birth; BBG2Na will also have to be capable of avoiding potential exacerbation of disease at time of later exposure to wild type RSV if used for neonatal immunization alone. The role of cell-mediated immunity to RSV after BBG2Na immunization will also be evaluated in terms of both protection and potential pathology enhancement. The distinct properties of BBG2Na make it an interesting RSV vaccine candidate for either or both of the two vaccine strategies currently under consideration in the hope of reducing the enormous burden of RSV disease in early life.

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References

1. Parrott RH, Kim HW, Arrobio JO, et al. Epidemiology of respiratory syncytial virus infection in Washington, DC. II. Infection and disease with respect to age, immunologic status, race, and sex. *Am J Epidemiol* 1973;98:289–300.
2. Martin AJ, Gardner PS, McQuillin J. Epidemiology of respiratory viral infection among pediatric inpatients over a six year period in northeast England. *Lancet* 1978;2:1035–8.
3. Hall CB, Kopelman AE, Douglas RG, et al. Neonatal respiratory syncytial virus infection. *N Engl J Med* 1979;300:393–6.
4. Glezen WP, Paredes A, Allison JE, et al. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group and maternal antibody level. *J Pediatr* 1981;140:543–6.
5. Green M, Brayer AF, Schenkman KA, Wald ER. Duration of hospitalization in previously well infants with respiratory syncytial virus infection. *Pediatr Infect Dis J* 1989;8:601–5.
6. Cane PA, Pringle CR. Evolution of subgroup A respiratory syncytial virus: evidence for progressive accumulation of amino acid changes in the attachment protein. *J Virol* 1995;69:2918–25.
7. Garcia O, Martin M, Dopazo J, et al. Evolutionary pattern of human respiratory syncytial virus (subgroup A): cocirculating lineages and correlation of genetic and antigenic changes in the G glycoprotein. *J Virol* 1994;68:5448–59.
8. Sullender WM, Mufson MA, Anderson LJ, Wertz GW. Genetic diversity of the attachment protein of subgroup B respiratory syncytial viruses. *J Virol* 1991;65:5425–34.
9. Chin J, Magoffin RL, Shearer LA, Schieble JH, Lennette EH. Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. *Am J Epidemiol* 1969;89:449–63.
10. Kim HW, Leikin SL, Arrobio JO, Brandt CD, Chanock RM, Parrott RH. Cell-mediated immunity to RSV induced by inactivated vaccine or by infection. *Pediatr Res* 1976;10:75–8.
11. Åkerlind-Stopner B, Utter G, Mufson MA, Orvell C, Lerner RA, Norrby E. A subgroup-specific antigenic site in the G protein of respiratory syncytial virus forms a disulfide-bonded loop. *J Virol* 1990;64:5143–8.
12. Trudel M, Nadon F, Seguin C, Binz H. Protection of BALB/c mice from respiratory syncytial virus infection by immunization with a synthetic peptide derived from the G glycoprotein. *Virology* 1991;185:749–57.
13. Nygren PÅ, Flodby P, Andersson R, Wigzell H, Uhlén M. In: Chanock RM, Brown F, Ginsberg HS, Lerner RA, eds. Vaccines 91, modern approaches to vaccine development. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1991:363–8.
14. Makrides SC, Nygren PÅ, Andrews B, et al. Extended in vivo half-life of human soluble complement receptor type 1 fused to a serum albumin-binding receptor. *J Pharmacol Exp Ther* 1996;277:534–42.
15. Sjölander A, Nygren PÅ, Ståhl S, et al. The serum albumin-binding region of streptococcal protein G: a bacterial fusion partner with carrier-related properties. *J Immunol Methods* 1997;201:115–23.
16. Wagner DK, Muelenaer P, Henderson FW, et al. Serum immunoglobulin G antibody subclass response to respiratory syncytial virus F and G glycoproteins after first, second, and third infections. *J Clin Microbiol* 1989;27:589–92.
17. Power UF, Plotnicky-Gilquin H, Huss T, et al. Induction of protective immunity in rodents by vaccination with a prokaryotically expressed recombinant fusion protein containing a respiratory syncytial virus G protein fragment. *Virology* 1997;230:155–66.
18. Murby M, Samuelsson E, Nguyen TN, et al. Hydrophobicity engineering to increase solubility and stability of a recombinant protein from respiratory syncytial virus. *Eur J Biochem* 1995;230:38–44.
19. Taylor G, Stott EJ, Hughes M, Collins AP. Respiratory syncytial virus infection in mice. *Infect Immun* 1984;43:649–55.
20. Barrios C, Brawand P, Berney M, Brandt C, Lambert PH, Siegrist CA. Neonatal and early life immune responses to various forms of vaccine antigens qualitatively differ from adult responses: predominance of a

- Th2-biased pattern which persists after adult boosting. *Eur J Immunol* **1996**;26:1489–96.
21. Hemming VG, Prince GA, Groothuis JR, Siber GR. Hyperimmune globulins in prevention and treatment of respiratory syncytial virus infections. *Clin Microbiol Rev* **1995**;8:22–33.
 22. Simister NE, Mostov KE. An Fc receptor structurally related to MHC class I antigens. *Nature* **1989**;337:184–7.
 23. Ahouse JJ, Hagerman CL, Mittal P, et al. Mouse MHC class I-like Fc receptor encoded outside the MHC. *J Immunol* **1993**;151:6076–88.
 24. Mackenzie NM, Keeler KD. A flow microfluorimetric analysis of the binding of immunoglobulin to Fcγ receptors on brush borders of the neonatal mouse jejunal epithelium. *Immunology* **1984**;51:529–33.
 25. Bangham CR. Passively acquired antibodies to respiratory syncytial virus impair the secondary cytotoxic T-cell response in the neonatal mouse. *Immunology* **1986**;59:37–41.
 26. Walsh EE, Hall CB, Briselli M, Brandriss MW, Schlesinger JJ. Immunization with glycoprotein subunits of respiratory syncytial virus to protect cotton rats against viral infection. *J Infect Dis* **1987**;155:1198–204.
 27. Routledge EG, Willcocks MM, Samson AC, et al. The purification of four respiratory syncytial virus proteins and their evaluation as protective agents against experimental infection in BALB/c mice. *J Gen Virol* **1988**;69:293–303.
 28. Murphy BR, Sotnikov A, Paradiso PR, et al. Immunization of cotton rats with the fusion (F) and large (G) glycoproteins of respiratory syncytial virus (RSV) protects against RSV challenge without potentiating RSV disease. *Vaccine* **1989**;7:533–40.
 29. Hancock GE, Speelman DJ, Frenchick PJ, Mineo-Kuhn MM, Baggs RB, Hahn DJ. Formulation of the purified fusion protein of respiratory syncytial virus with the saponin QS-21 induces protective immune responses in BALB/c mice that are similar to those generated by experimental infection. *Vaccine* **1995**;13:391–400.
 30. Elango N, Prince GA, Murphy BR, Venkatesan S, Chanock RM, Moss B. Resistance to human respiratory syncytial virus (RSV) infection induced by immunization of cotton rats with a recombinant vaccinia virus expressing the RSV G glycoprotein. *Proc Natl Acad Sci USA* **1986**;83:1906–10.
 31. Stott EJ, Ball LA, Young KK, Furze J, Wertz GW. Human respiratory syncytial virus glycoprotein G expressed from a recombinant vaccinia virus vector protects mice against live-virus challenge. *J Virol* **1986**;60:607–13.
 32. Johnson PR Jr, Olmsted RA, Prince GA, et al. Antigenic relatedness between glycoproteins of human respiratory syncytial virus subgroups A and B: evaluation of the contributions of F and G glycoproteins to immunity. *J Virol* **1987**;61:3163–6.
 33. Stott EJ, Taylor G, Ball LA, et al. Immune and histopathological responses in animals vaccinated with recombinant vaccinia viruses that express individual genes of human respiratory syncytial virus. *J Virol* **1987**;61:3855–61.
 34. Olmsted RA, Murphy BR, Lawrence LA, Elango N, Moss B, Collins PL. Processing, surface expression, and immunogenicity of carboxy-terminally truncated mutants of G protein of human respiratory syncytial virus. *J Virol* **1989**;63:411–20.
 35. Ridge JP, Fuchs EJ, Matzinger P. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* **1996**;271:1723–6.
 36. Sarzotti M, Robbins DS, Hoffman PM. Induction of protective CTL responses in newborn mice by a murine retrovirus. *Science* **1996**;271:1726–8.
 37. Forsthuber T, Yip HC, Lehmann PV. Induction of TH1 and TH2 immunity in neonatal mice. *Science* **1996**;271:1728–30.
 38. Singh RR, Hahn BH, Sercarz EE. Neonatal peptide exposure can prime T cells and, upon subsequent immunization induce their immune deviation: implications for antibody versus T cell-mediated autoimmunity. *J Exp Med* **1996**;183:1613–21.
 39. Barrios C, Brandt C, Berney M, Lambert PH, Siegrist CA. Partial correction of the TH1/TH2 imbalance in neonatal murine responses to vaccine antigens through selective adjuvant effects. *Eur J Immunol* **1996**; 26:2666–70.
 40. Belshe RB, van Voris LP, Mufson MA. Parenteral administration of live respiratory syncytial virus vaccine: results of a field trial. *J Infect Dis* **1982**;145:311–9.
 41. Murphy BR, Alling DW, Snyder MH, et al. Effect of age and preexisting antibody on serum antibody response of infants and children to the F and G glycoproteins during respiratory syncytial virus infection. *J Clin Microbiol* **1986**;24:894–8.
 42. Prince GA, Horswood RL, Camargo E, Suffin SC, Chanock RM. Parenteral immunization with live respiratory syncytial virus is blocked in seropositive cotton rats. *Infect Immun* **1982**;37:1074–8.
 43. Murphy BR, Olmsted RA, Collins PL, Chanock RM, Prince GA. Passive transfer of respiratory syncytial virus (RSV) antiserum suppresses the immune response to the RSV fusion (F) and large (G) glycoproteins expressed by recombinant vaccinia viruses. *J Virol* **1988**;62:3907–10.
 44. Murphy BR, Prince GA, Collins PL, Hildreth SW, Paradiso PR. Effect of passive antibody on the immune response of cotton rats to purified F and G glycoproteins of respiratory syncytial virus (RSV). *Vaccine* **1991**; 9:185–9.